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14. ABSTRACT
This study proposed to evaluate contrast-enhanced ultrasound imaging with a microbubble contrast agent in 300 subjects in order to improve the detection of prostate cancer. Between October 2001 and January 2004 a total of 301 subjects were enrolled. Laboratory blood tests (PSA) and ultrasound evaluations were completed on all 301 subjects (including the primary ultrasound interpretation worksheet). Independent blinded readers have reviewed imaging on all subjects. Pathological review for the presence and grade of cancer has been completed for all subjects. CD31 staining for micro-vessel density has been performed on the first 40 subjects. All available data has been entered into a computer database using an Excel spreadsheet. Analysis of the prostate cancer detection data from all 301 subjects was incorporated into a manuscript that has been accepted for publication in the journal "Cancer". The final analysis of microvessel density has been accepted for presentation at the annual meeting of the Radiological Society of North America (Dec 2005). Copies of the manuscript and the abstract are appended to this report.

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INTRODUCTION:

Numerous studies have demonstrated an increased number of very small blood vessels in prostate cancers, as well as an association between the number of these vessels and aggressiveness of disease. The present study is designed to visualize these vessels with ultrasound during intravenous infusion of a microbubble contrast agent. The objective of this study is to utilize contrast-enhanced ultrasound imaging to improve the detection of prostate cancer, in order to identify those cancers which are clinically significant.

The study protocol includes enrollment of three hundred subjects with suspected cancer of the prostate. Each subject is imaged with conventional and intermittent ultrasound both before and after administration of the contrast agent. Based upon a comparison of ultrasound findings with biopsy results, this study is designed to demonstrate that contrast-enhanced intermittent ultrasound imaging of the prostate results in improved detection of prostate cancer. Furthermore, ultrasound findings with the contrast agent are to be correlated with microvessel density, Gleason score and PSA in order to determine whether intermittent imaging can selectively identify clinically significant cancers.

BODY:

Statement of Work tasks:

#1 - Ultrasound contrast studies:

Patient recruitment was completed in January 2004. A total of 301 subjects provided written informed consent, were evaluated with the required laboratory studies (PSA) and participated in the ultrasound contrast protocol. The examining physician (Dr. Ethan Halpern) has completed an ultrasound image interpretation worksheet for each of these subjects. This portion of the study was completed as scheduled.

#2 – Pathologic evaluation:

Prostate biopsy specimens were obtained from all 301 subjects and were evaluated by standard pathologic evaluation. A pathology interpretation worksheet has been completed by our pathology consultant (Dr. Peter McCue).

For the evaluation of microvessel density, CD31 staining has been performed on tissue sections from 40 subjects, and stained specimens have been evaluated for 15 subjects. Due to a variety of technical difficulties beyond our control, the analysis process for microvessel density took much longer than expected. Microvessel density assessment is performed on a complex computer-based histomorphology system. A computer failure last year resulted in loss of the vessel counting software and made the entire system inaccessible for almost five months. An alternate source of funding was identified to repair the computer system and purchase new vessel counting software. During the one year extension of this grant, the computer system was repaired and a replacement graduate student was identified to complete the project. CD31 microvessel density has now been completed on a total of 15 subjects. The entire process of microvessel density counting was much more tedious than originally anticipated. An abstract of the microvessel density work has been accepted for presentation at the annual meeting of the Radiological Society of North America in Dec '05. The data on our 15 subjects will be presented at that time, but is similar to the data reported for the initial 13 subjects reported in the abstract (see appendix).

#3 – Database entry:

A database has been established. All ultrasound, laboratory and pathology data have been entered into the database by the research coordinator.

#4 – Interim statistical evaluation:

The interim evaluation was reported in the previous annual report. Several abstracts reporting the interim analysis were presented at the annual meeting of the Radiological Society of North America in 2003. These abstracts were included in the previous annual report. Additional abstracts were presented at the annual meeting of the Radiological

Society of North America in 2004. These are included in the appendix of this report. References to all of these abstracts are provided in the reference section below. Statistical analysis of the data for the entire study is presented in the final paper (now in-press in the journal "Cancer") included in the appendix.

#5 – Blinded reader & consensus interpretations:

In addition to the observations recorded by the primary reader, independent observations by a blinded observer have been completed during the one year extension of this grant for all subjects. This data is included in the final paper included in the appendix.

#6 – Analysis & Publications:

The final results of this study have now bee accepted for publication in "Cancer". A brief review of the results and conclusions is presented below. A full reference to this manuscript is in the reference section below. The full text of the manuscript is in the appendix.

Results: Cancer was detected in 363 biopsy cores from 104 of 301 subjects (35%). Cancer was found in 15.5% (175/1133) of targeted cores and 10.4% (188/1806) of sextant cores ($p < 0.01$). Among subjects with cancer, targeted cores were twice as likely to be positive (OR = 2.0, $p < 0.001$). Clustered ROC analysis of imaging findings at sextant biopsy sites yielded the following Az values: pre-contrast gray scale – 0.58, pre-contrast color Doppler – 0.53, pre-contrast power Doppler – 0.58, CHI – 0.62, IHI (0.2s) – 0.64, IHI (0.5s) – 0.63, IHI (1.0s) – 0.65, IHI (2.0s) – 0.61, contrast-enhanced color Doppler – 0.60, contrast-enhanced power Doppler – 0.62. A statistically significant benefit was found for IHI over baseline imaging ($p < 0.05$).

Conclusions: The cancer detection rate of contrast-enhanced targeted cores is significantly higher when compared to sextant cores. Contrast-enhanced transrectal sonography with IHI provides a statistically significant improvement in discrimination between benign and malignant biopsy sites. However, given relatively low ROC areas, this technique may not be sufficient to predict which patients have benign versus malignant disease.

KEY RESEARCH ACCOMPLISHMENTS:

- Successful infusion of ultrasound contrast in 301 subjects with ultrasound guided biopsy.
- Targeted cores, based upon ultrasound findings with contrast-enhanced imaging, detected the presence of prostate cancer twice as frequently as non-targeted cores (OR = 2.0, $p < 0.001$).
- Contrast-enhanced intermittent harmonic imaging provided a statistically significant ($p < 0.05$) improvement in discrimination between benign and malignant areas of the prostate outer gland .
- Targeted biopsy based upon contrast enhancement detected an additional 11 patients with cancer that would not have been detected with the conventional sextant biopsy protocol.
- Microvessel density was found to be greater in malignant than in normal prostate tissues ($p = 0.0009$);
- Microvessel density correlated with the enhancement seen on contrast-enhanced ultrasound imaging ($r=0.24$; $p=0.0166$).

REPORTABLE OUTCOMES: One new abstracts accepted for presentation. The manuscript describing our final results is in-press in "Cancer".

- Forsberg F; Yu J; Kuruvilla B; Halpern EJ. Contrast-enhanced TRUS and Microvessel Density Correlates in Prostate Cancer. Accepted for presentation at the annual meeting of the RSNA – Dec 2005 (text of abstract is attached in the appendix).

- Halpern EJ, Ramey JR, Strup SE, Frauscher F, McCue P, Gomella LG. Detection of Prostate Cancer with Contrast Enhanced Sonography Using Intermittent Harmonic Imaging. Cancer – in press. (full text is attached at the end of the appendix)

CONCLUSIONS:

       Intravenous infusion of a microbubble contrast agent provides sonographically visible enhancement of the prostate. This enhancement can be used to guide biopsy of the prostate into areas of increased vascular flow. Among subjects with cancer, targeted cores were twice as likely to return a positive biopsy (OR = 2.0, $p < 0.001$). With respect to the characterization of tissue as benign versus malignant, a statistically significant benefit was found for all methods of post-contrast intermittent harmonic imaging over baseline gray scale and Doppler imaging ($p < 0.05$). Targeted biopsy of the prostate based upon contrast-enhanced imaging will identify cancers that are not detected by conventional sextant biopsy. However, targeted biopsy will also miss cancers that might be detected by a systematic sextant biopsy. As noted in our prior report, most cancers that were not identified with the targeted contrast-enhanced technique were located at the apex of the gland. In order to maximize cancer detection, we therefore recommend a contrast-enhanced targeted biopsy strategy with additional systematic cores distributed to the apex of the prostate.

REFERENCES:

Forsberg F; Yu J; Kuruvilla B; Halpern EJ. Contrast-enhanced TRUS and Microvessel Density Correlates in Prostate Cancer. Accepted for presentation at the annual meeting of the RSNA – Dec 2005 (text of abstract is attached in the appendix).

Halpern EJ, Frauscher F, Ramey JR, McCue P, Gomella LG. Prostate Cancer Detection with Targeted Biopsy during Contrast-enhanced Sonography. Proceedings of the 2004 meeting of the RSNA. Pg 268, December 2004.

Halpern EJ, Ramey JR, Frauscher F, McCue P,Gomella LG. Detection of Prostate Cancer with Contrast-enhanced Sonography Using Harmonic Gray Scale, Color Doppler, and Power Doppler Imaging. Radiology: Proceedings of the 2004 meeting of the RSNA. Pg 269, December 2004.

Halpern EJ, Ramey JR, Strup SE, Frauscher F, McCue P, Gomella LG. Detection of Prostate Cancer with Contrast Enhanced Sonography Using Intermittent Harmonic Imaging. Cancer – in press. (full text is attached at the end of the appendix)

Previously reported abstracts:

Forsberg F, Kuruvilla B, Elwood D, Halpern EJ. Microvessel density and contrast enhanced TRUS for prostate cancer diagnosis. Journal of Ultrasound in Medicine 23(suppl): S53, 2004.

Halpern EJ, Frauscher F, Strup SE, Ramey JR, Gomella LG. Comparison of Contrast-enhanced Targeted Biopsy of the Prostate to Modified Sextant Biopsy. Radiology: Proceedings of the 2003 meeting of the RSNA. Pg 666, December 2003.

Halpern EJ, Frauscher F, Strup SE, Ramey JR, Gomella LG. Contrast Enhanced Imaging of the Prostate for Cancer Detection. Radiology: Proceedings of the 2003 meeting of the RSNA. Pg 665, December 2003.

Halpern EJ, Strup SE, Ramey JR, Gomella LG. Comparison of Contrast-enhanced Targeted Biopsy of the Prostate to Modified Sextant Biopsy. Proceedings of the 61st annual meeting of the Mid-Atlantic Section of the American Urological Association. Pg 114, Boca Raton, Fl, Oct 2003.

APPENDICES:

Accepted for presentation in Dec '05 to the Radiological Society of North America

Thomas Jefferson University
Dept of Radiology (763-J)
Primary Category: Ultrasound
Secondary Category: Contrast Agents
Contrast-enhanced TRUS and Microvessel Density Correlates in Prostate Cancer
F Forsberg, PhD, Philadelphia, PA; J Yu; B Kuruvilla; E J Halpern, MD
(flemming.forsberg@jefferson.edu)

PURPOSE
To evaluate microvessel density (MVD) measurements and contrast enhanced transrectal ultrasound imaging (TRUS), using the US contrast agent Imagent® (IMCOR Pharmaceutical, San Diego, CA), in prostate cancer.

METHOD AND MATERIALS
Thirteen men scheduled for TRUS guided prostate biopsies (random sextant biopsies and up to 4 additional contrast directed biopsies) were evaluated. TRUS was performed after infusion of Imagent (approximate dose 0.31 mg/{kg min}) in grayscale phase inversion harmonic imaging (PIHI), color Doppler imaging (CDI) and power Doppler imaging (PDI) modes using an Elegra scanner (Siemens Medical Systems, Issaquah, WA). The enhancement and the suspicion of cancer at each biopsy site were assessed prospectively on a 5-point scale. All biopsy specimens were assessed for cancer with standard H&E stain and for MVD with an endothelial cell marker stain (CD31). MVD was determined using an SMZ-10A microscope (magnification 100x; Nikon, Melville, NY) and Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The area under the receiver operating characteristic (ROC) curve for MVD was computed using histopathology as the gold standard. Linear regression was used to correlate MVD with enhancement and suspicion of cancer for all imaging modes.

RESULTS
Of the 121 biopsy specimens, 99 had sufficient tissue for MVD determination. Sixteen (16%) contained malignant prostate tissue. The area under the ROC curve for the diagnosis of prostate cancer with MVD was 0.77. There was a statistically significant difference between benign and malignant MVDs (mean and standard deviations: 30.6 ± 26.29 and 67.3 ± 78.22 vessels/mm^2, respectively; $p=0.0009$). MVD correlated significantly with PIHI enhancement ($r=0.24$; $p=0.0166$) but not with CDI and PDI enhancement ($r<0.14$; $p>0.17$). The suspicion of cancer assessed with all 3 TRUS modes correlated significantly with MVD ($r>0.33$; $p<0.0007$).

CONCLUSION
MVD is greater in malignant than in normal prostate tissues. MVD correlates with the enhancement seen on grayscale PIHI TRUS demonstrating the angiogenic underpinnings of PIHI of prostate cancer. This work was supported in part by DAMD17-01-1-0061 and IMCOR Pharmaceutical, San Diego, CA.

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PROSTATE CANCER DETECTION WITH TARGETED BIOPSY DURING CONTRAST ENHANCED SONOGRAPHY
E J Halpern (P); F Frauscher; J R Ramey; P McCue; L G Gomella

PURPOSE
To evaluate cancer detection with a contrast-enhanced targeted biopsy approach compared with a modified sextant biopsy distribution.

METHOD AND MATERIALS
Three hundred and one subjects with an elevated PSA (above 4ng/ml) or abnormal digital rectal examination were evaluated by transrectal sonography during infusion of a microbubble contrast agent (Imagent; Imcor). Sonography was performed with a 6.5MHz end-fire transducer. Up to four targeted biopsy cores were obtained from the sites of greatest enhancement in the outer gland during contrast-enhanced imaging. Six additional outer gland biopsy cores were obtained in a modified sextant distribution.

RESULTS
Cancer was detected in 363 biopsy cores from 104 of 301 subjects (35%), including 15.5% (175/1133) of targeted cores and 10.4% (188/1806) of sextant cores (p < 0.01). Among subjects with cancer, targeted cores were twice as likely to return a positive biopsy (OR = 2.0, p < 0.001). Cancer was discovered in 72 subjects by both techniques, in 21 subjects by sextant biopsy alone and in 11 subjects by targeted biopsy alone (p = 0.08). The 21 subjects with cancer detected by sextant biopsy alone included 5 positive cores at the gland base, 7 in the mid-gland and 17 in the apex. The 11 subjects with cancer detected by targeted biopsy alone included 8 positive cores at the gland base, 4 in the mid-gland and 3 in the apex. While 38% (72/188) of positive sextant cores were obtained at the gland apex, only 17% (30/175) of positive targeted cores were obtained from the gland apex. Only 21% (233/1133) of targeted biopsies were directed to the apex.

CONCLUSIONS
The cancer detection rate of contrast-enhanced targeted cores is significantly higher when compared to a modified sextant approach. Although targeted biopsy detected 11% (11/104) of cancers not found by the sextant approach, targeted biopsy failed to detect 20% (21/104) of cancers. The low proportion of targeted biopsy cores at the apex suggests that contrast enhancement is less efficacious at the apex. In order to maximize cancer detection and minimize the number of biopsy cores, we recommend a contrast-enhanced targeted biopsy strategy with additional cores at the apex of the prostate.

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DETECTION OF PROSTATE CANCER WITH CONTRAST ENHANCED SONOGRAPHY USING HARMONIC GRAY SCALE, COLOR DOPPLER AND POWER DOPPLER IMAGING
E J Halpern (P); J R Ramey; F Frauscher; P McCue; L G Gomella

PURPOSE
To evaluate the discrimination of benign from malignant prostate outer gland tissue during contrast-enhanced sonography.

METHOD AND MATERIALS
301 subjects with an elevated PSA or abnormal digital rectal examination were evaluated with transrectal sonography during infusion of a microbubble contrast agent (Imagent; Imcor). Baseline imaging was performed with conventional gray scale, color and power Doppler. Contrast-enhanced imaging was performed with harmonic gray scale, including continuous harmonic imaging (CHI) and intermittent haramonic imaging (IHI) with interscan delay times of 0.2s, 0.5s, 1.0s, 2.0s, as well as with continuous color and power Doppler. Six biopsy cores were obtained in a modified sextant distribution with one core from the most suspicious area in each sextant. A sextant with no suspicious area was sampled with a laterally directed core. Each biopsy site was prospectively rated for suspicion of cancer on a 1-5 scale with each imaging technique. In order to compensate for clustering of data within each subject, clustered ROC analysis was performed.

RESULTS
Cancer was detected in 188 sextant cores from 93 of 301 subjects (31%). Clustered ROC analysis demonstrated the following values for area under the curve, Az: pre-contrast gray scale – 0.58, pre-contrast color Doppler – 0.53, pre-contrast power Doppler – 0.58, CHI – 0.62, IHI (0.2s) – 0.64, IHI (0.5s) – 0.63, IHI (1.0s) – 0.65, IHI (2.0s) – 0.61, contrast-enhanced color Doppler – 0.60, contrast enhanced power Doppler – 0.62. A statistically significant benefit was found for IHI over baseline gray scale and Doppler imaging ($p < 0.05$).

CONCLUSIONS
Contrast-enhanced transrectal sonography with IHI provides a statistically significant improvement in discrimination between benign and malignant areas of the prostate outer gland. However, as evidenced by relatively low ROC areas, contrast enhanced sonography cannot definitively differentiate benign from malignant tissue without biopsy confirmation.

DETECTION OF PROSTATE CANCER WITH CONTRAST ENHANCED SONOGRAPHY USING INTERMITTENT HARMONIC IMAGING

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Abstract:

Purpose: To assess prostate cancer detection and discrimination of benign from malignant prostate tissue with contrast-enhanced ultrasonography.

Methods and Materials: 301 subjects referred for prostate biopsy were evaluated with contrast-enhanced sonography using continuous harmonic imaging (CHI) and intermittent harmonic imaging (IHI) with interscan delay times of 0.2s, 0.5s, 1.0s, 2.0s, as well as continuous color and power Doppler. Targeted biopsy cores were obtained from sites of greatest enhancement, followed by spatially distributed cores in a modified sextant distribution.

Results: Cancer was detected in 363 biopsy cores from 104 of 301 subjects (35%). Cancer was found in 15.5% (175/1133) of targeted cores and 10.4% (188/1806) of sextant cores ($p < 0.01$). Among subjects with cancer, targeted cores were twice as likely to be positive ($OR = 2.0$, $p < 0.001$). Clustered ROC analysis of imaging findings at sextant biopsy sites yielded the following Az values: pre-contrast gray scale – 0.58, pre-contrast color Doppler – 0.53, pre-contrast power Doppler – 0.58, CHI – 0.62, IHI (0.2s) – 0.64, IHI (0.5s) – 0.63, IHI (1.0s) – 0.65, IHI (2.0s) – 0.61, contrast-enhanced color Doppler – 0.60, contrast-enhanced power Doppler – 0.62. A statistically significant benefit was found for IHI over baseline imaging ($p < 0.05$).

Conclusion: The cancer detection rate of contrast-enhanced targeted cores is significantly higher when compared to sextant cores. Contrast-enhanced transrectal sonography with IHI provides a statistically significant improvement in discrimination between benign and malignant biopsy sites. However, given relatively low ROC areas, this technique may not be sufficient to predict which patients have benign versus malignant disease.

Introduction:

The number of new cases of prostate cancer that will be diagnosed in the United States for 2005 is estimated at 232,090 with 30,350 deaths.[i] Between 1986 and 1991 the rate of prostate needle biopsy in men over 65 years of age increased from 685 to 2600 per 100,000.[ii] Since the proportion of prostate biopsies positive for cancer is slightly under one-third, the number of prostate biopsies performed annually in the United States in 2005 is estimated to be greater than 700,000.

The sextant biopsy protocol, a systematic, spatially distributed set of six biopsy cores obtained under transrectal ultrasound guidance, was described in 1989, and remained the standard of care for a decade.[iii] More recently, clinicians have begun to advocate 10-12 biopsy cores[iv,v] or a "saturation biopsy" approach.[vi] Such systematic techniques increase the volume of sampled tissue, but do not identify and target specific lesions. Since patient morbidity and pathology costs are related to the number of biopsy cores, a targeted biopsy approach that could maintain the efficiency of cancer detection with a reduced number of biopsy cores would represent a cost-effective approach to the diagnosis of prostate cancer.

Cancer of the prostate is classically described as hypoechoic,[vii] but can appear echogenic or isoechoic.[viii] Color Doppler imaging has been proposed to supplement conventional gray scale imaging,[ix,x] Increased color Doppler signal correlates positively with both prostate tumor stage and grade, as well as with the risk of recurrence after treatment.[xi] Power Doppler may be even more useful in the detection of prostate

cancer.[xii,xiii] Nonetheless, conventional color and power Doppler guided needle biopsy do not substantially improve the detection rate of prostate cancer.[xiv] The combination of gray scale and Doppler ultrasound is not sufficient to eliminate the need for systematic biopsy.[xv,xvi,xvii]

Microbubble contrast agents enhance sonographic visualization of the microvasculature associated with prostate cancer.[xviii,xix] These agents increase the echogenicity of the intravascular space on gray scale harmonic imaging, and also provide a dramatic visible increase in Doppler signal. Intermittent imaging is an ultrasound technique that employs a reduced frame rate, allows more time for contrast agent to enter the scan plane between frames, and thereby increases the intensity of microbubble contrast enhancement.[xx,xxi,xxii] Preliminary data suggest that intermittent gray scale harmonic imaging (IHI) can increase the conspicuity of microvascular enhancement associated with prostate cancer.[xxiii] The current study was designed to evaluate the ability of contrast-enhanced sonography with intermittent gray scale harmonic imaging to improve the detection of prostate cancer with a targeted biopsy technique.

Methods

Study Population

IRB approval was obtained for this Department of Defense sponsored protocol, and written informed consent was obtained from each participant. Three hundred and one subjects with an elevated prostate specific antigen (PSA ≥4ng/ml) or abnormal digital rectal examination were enrolled between October 2001 and January 2004. Mean patient age was 63 +/-8 with a range of 42-87. Mean PSA was 9.5ng/ml +/-23 with a range of 0.4-360.7. There were 73 subjects with a PSA below 4.0ng/ml, 118 subjects with a PSA above 4 and below 10, 54 subjects with a PSA in the range of 10-35, and 6 subjects with PSA above 35. The population consists of 232 Caucasian males, 52 African American males, 3 Indian males (from India), 8 Asian males, 2 Philippine males, 3 Hispanic males and 1 Asian/Hispanic male. Just under half of the study subjects (n=134) had a previous negative biopsy procedure of the prostate with a PSA that remained elevated.

Imaging Protocol

Sonography was performed with the Sonoline Elegra system (Siemens Medical Systems; Issaquah, Wash) using a 6.5MHz end-fire transducer. In order to reduce the impact of patient position on prostatic blood flow, all subjects were examined in the lithotomy position.[xxiv] Gray scale imaging was performed with a center probe frequency of 5.14 MHz, a dynamic range of 55dB and a persistence setting of 2. Continuous gray scale harmonic imaging (CHI) was performed with a default mechanical index of 0.4. The mechanical index was automatically increased into the range of 0.8-1.0 for IHI. For color and power imaging the center probe frequency was at 4.0MHz with a dynamic range of 30dB, pulse repetition frequency of 868Hz, and wall filter set to low. The

Doppler window for color and power imaging included the entire gland. Color and power gain were adjusted to maximize signal but eliminate color noise from the tissue of the prostate. The entire examination was recorded on sVHS videotape.

In order to obtain comparable images from the pre-contrast and post-contrast portions of the examination, multiple identical series of angled axial sweeps through the gland were obtained from base to apex, each sweep extending over a period of 20-30 seconds. Pre-contrast imaging sweeps were performed with conventional gray scale imaging as well as gray scale harmonic imaging, color Doppler and power Doppler. Post contrast imaging sweeps were performed with gray scale harmonic imaging in continuous mode, and repeated during intermittent harmonic imaging (IHI) with interscan delay times of 0.2seconds, 0.5seconds, 1.0seconds and 2.0seconds. Two additional post contrast imaging sweeps were performed with continuous color Doppler and continuous power Doppler imaging.

Contrast Infusion

Contrast enhanced imaging was performed during infusion of AF0150 (*Imagent*®, formerly: Alliance Pharmaceutical Corp.; San Diego, CA now: Imcor; San Diego, CA). AF0150 is a sterile, non-pyrogenic white to off-white powder of spray-dried microspheres. The microspheres consist of surfactants, buffers, salts, and a water-soluble structural agent that dissolve when reconstituted, forming a dispersion of stable and highly echogenic microbubbles (typical volume-weighted mean diameter of 6 μm)

in a buffered, iso-osmotic solution. AFO150 remains within the circulation for several minutes after injection, and produces both gray scale and Doppler enhancement.[xxv]

Based upon information gained in the previous trials of Imagent, contrast material was delivered by intravenous infusion during the prostate examination and biopsy procedure. A dose of 4.0mg/kg of Imagent was added to 150cc of normal saline. For the average patient, this dose amounted to two patient contrast kits with a retail cost of $250 ($125 per patient kit). The initial infusion rate was 8cc/minute. Post contrast imaging began as soon as the contrast was visible on continuous gray scale imaging. The infusion rate was adjusted in the range of 8-12cc/minute in order to subjectively optimize visible enhancement of the prostate. The infusion continued for approximately 10 minutes during which time imaging sweeps and biopsy were performed.

Biopsy Protocol

After the completion of contrast-enhanced imaging, ultrasound guided biopsies was performed with an 18-gauge automated spring-loaded biopsy gun. Topical anesthesia was given with lidocaine jel, but a transrectal injection of lidocaine was not performed because of concerns related to possible effects of injected lidocaine on blood flow within the prostate. Up to 4 targeted biopsy specimens per prostate (per patient) were first obtained from areas with the greatest amount of contrast enhancement, followed by a modified sextant biopsy. The modified sextant cores were obtained from the areas of greatest flow in the outer gland at the base, mid-portion and apex, on each the right and left sides. These cores were often laterally directed and often overlapped with the directed cores. When an area of increased flow was not identified within a

particular sextant, a laterally directed core was obtained for the modified sextant protocol. Thus, six modified sextant cores were obtained from each subject along with 0-4 targeted cores.

Image Interpretation:

A subjective rating score was assigned for each sextant biopsy site on each imaging sequence. Rating scores were assigned by the examining physician at the time of the initial examination. An independent interpretation of the imaging findings was performed by a second physician who reviewed a videotape of the examination, but was blinded to the initial interpretation as well as to all clinical and pathological information. The examining physician was an experienced radiologist who had performed many previous studies with contrast-enhanced ultrasound of the prostate. An experienced urologist who had performed many prostate biopsy procedures, but had not previously worked with contrast-enhanced ultrasound, performed the independent, blinded interpretations for the first 100 subjects. A junior urologist with little prior biopsy experience trained together with the examining physician during performance of the first 100 studies. This junior urologist performed the remaining independent blinded interpretations.

For baseline gray scale imaging, the suspicion of cancer was scored based upon tissue echotexture and gland contour abnormalities. For baseline color and power Doppler, the score was based upon level of Doppler flow observed. For post-contrast

imaging, each sextant biopsy site was scored for the level of contrast enhancement. A five

point subjective scale was used with the following general guidelines:

Baseline gray scale scoring System:

      1 – Normal appearance (homogeneous, echogenic outer gland)
      2 – Probably normal (minimal heterogeneity of the outer gland)
      3 – Indeterminate (contour asymmetry or ill-defined echotexture abnormality)
      4 – Probably cancer (focal contour bulge or probable mass)
      5 – Definitely cancer (focal hypoechoic mass)

Baseline Doppler scoring System:

      1 – Normal appearance (capsular & periurethral flow only)
      2 – Probably normal (symmetric radial flow extending in from capsular branches)
      3 – Indeterminate (subtle asymmetric/increased flow pattern in outer gland)
      4 – Probably cancer (definite asymmetric/increased flow in outer gland)
      5 – Definitely cancer (focal asymmetric/increased flow with disorganized pattern)

Contrast-enhanced scoring System:

      1 – Minimal enhancement (capsular & periurethral flow only)
      2 – Mild enhancement (symmetric radial flow from capsular branches)
      3 – Mildly increased enhacement (asymmetric/increased flow in prostate)
      4 – Moderately increased enhancement (asymmetric/increased flow in prostate)
      5 – Substantially increased enhancement (asymmetric/increased flow in prostate)

Analysis

The number of cancers found and the percentage of positive cores were tabulated

for both the targeted biopsy cores and the modified sextant cores (which are also targeted

within each sextant). These results were further stratified by the number of previous

biopsy procedures.

In order to compare the "by-core" positive biopsy yield of the targeted technique

to the positive yield of the modified sextant technique, conditional logistic regression

analysis was performed. Conditional logistic analysis was chosen because of clustered

(non-independent) biopsy sites within each subject. An odds ratio and corresponding confidence interval were computed for the detection of cancer in targeted versus sextant biopsy cores (STATA 8; Stata Corporation, College Station, TX).

In order to compare the "by-patient" detection rate of cancer for targeted and sextant biopsy techniques, cancer detection with targeted and sextant techniques was tabulated by patient. A McNemar's chi-square was computed to compare the cancer detection rate with the two techniques (STATA 8; Stata Corporation, College Station, TX). In order to determine whether there was a difference in location of cancer detected by targeted versus sextant biopsy, the number of positive cores obtained from the base, mid-gland and apex was tabulated, and a chi-square test for trend was performed (Epi-Info 6; Centers for Disease Control & Prevention, Atlanta, GA). In order to determine whether the targeted technique detected higher grade cancers, the Gleason scores of cancers detected by targeted and sextant biopsy were tabulated, and compared with a chi-square test for trend (Epi-Info 6; Centers for Disease Control & Prevention, Atlanta, GA).

Receiver operating characteristic (ROC) analysis was used to assess the diagnostic accuracy of ultrasound for the detection of prostate cancer based upon the 5-point subjective scores at each sextant biopsy site. In order to compensate for the lack of independence among the sextant biopsy sites within an individual patient, clustered ROC analysis was performed.[xxvi] In order to avoid the bias that would result from redundant biopsies at sites with greater enhancement, only the sextant biopsy data was used for the clustered ROC analysis.

In order to determine whether contrast-enhanced ultrasound and targeted biopsy would selectively detect additional forms of prostate pathology, biosy cores with a finding of prostatic intraepithelial neoplasia (PIN), atypical small acinar proliferation (ASAP) and prostatitis were identified. Conditional logistic regression was used to determine whether there was a statistically significant increased probability of detecting these three types of prostate pathology with targeted biopsy.

In order to evaluate interobserver agreement, a kappa score was computed to quantify interobserver agreement between the rating scores of the examining physician and each of the two blinded readers. A quadratic weighted kappa was used to accommodate the five point rating score for each biopsy site (STATA 8; Stata Corporation, College Station, TX).

Results:

Infusion of contrast material resulted in visible vascular enhancement in every subject. Total examination time for the baseline and contrast-enhanced study was extended by about 15 minutes compared to the time required for a non-contrast study. One patient experienced a delayed allergic reaction which may have been related to antibiotic prophylaxis. Another patient experienced a severe vasovagal episode which appeared to be related to the biopsy procedure. No adverse events related to the contrast agent were observed.

Illustrations of contrast-enhanced imaging of the prostate are presented in figures 1 and 2. Figure 1 demonstrates a hypoechoic Gleason 8 cancer on baseline imaging that

demonstrates contrast enhancement. Figure 2 demonstrates another Gleason 8 cancer that is not clearly defined with baseline gray scale or Doppler imaging, but is clearly enhanced on post-contrast gray scale harmonic and Doppler imaging. The tumor blush appears qualitatively different with IHI as compared to continuous harmonic gray scale imaging.

Cancer was detected in 363 biopsy cores from 104 of 301 subjects (35%). The positive biopsy rate is tabulated as a function of the number of previous biopsy procedures in table 1. Cancer was found in 15.5% (175/1133) of targeted cores and 10.4% (188/1806) of sextant cores. Among subjects with cancer, targeted cores were twice as likely to return a positive biopsy (logistic regression OR = 2.0, p < 0.001).

The diagnosis of cancer was discovered in 72 subjects by both targeted and sextant techniques. In 21 subjects cancer was detected by sextant biopsy alone and in 11 subjects cancer was detected by targeted biopsy alone (table 2). A "by-patient" McNemar analysis demonstrated no statistically significant advantage to the targeted versus the sextant approach (p = 0.08).

The 21 subjects with cancer detected by sextant biopsy alone included 5 cancers at the gland base, 7 in the mid-gland and 17 in the apex. The 11 subjects with cancer detected by targeted biopsy alone included 8 cancers at the gland base, 4 in the mid-gland and 3 in the apex. While 38% (72/188) of positive sextant cores were obtained at the gland apex, only 17% (30/175) of positive targeted cores were obtained from the gland apex. Chi square for trend analysis confirms a statistically significant trend to find more cancers

at the gland base with targeted cores and more cancers at the gland apex with systematic sextant cores (p = 0.006).

The distribution of Gleason scores in targeted and sextant cores in summarized in table 3. Chi square for trend analysis fails to demonstrate a significant relationship between biopsy technique and Gleason score (p = 0.36).

In order to evaluate the ability of contrast-enhanced ultrasound to discriminate between benign and malignant tissue, clustered ROC analysis of sextant biopsy specimens was performed (table 4). Cancer was detected in 188 sextant cores from 93 of 301 subjects (31%). ROC areas for baseline gray scale and Doppler imaging ranged from $A_z = 0.53$-0.58. ROC areas for contrast-enhanced imaging ranged from $A_z = 0.60$-0.65. A statistically significant benefit was found for all methods of post-contrast intermittent harmonic imaging over baseline gray scale and Doppler imaging (p < 0.05). No significant difference in ROC area was observed with contrast-enhanced imaging at different interscan delay times. No single intermittent delay time was significantly superior for characterization of malignant sites. Furthermore, there was no statistically significant advantage for intermittent contrast-enhanced imaging beyond that provided by continuous harmonic imaging. Although there was a statistically significant improvement in the characterization of tissue as benign versus malignant with contrast-enhanced imaging, the relatively low ROC areas (< 0.65) suggest that contrast enhanced sonography did not definitively differentiate benign from malignant tissue without biopsy confirmation.

The diagnosis of prostatitis was made in 352 biopsy cores, including 158/1133 (13.9%) of targeted cores and 194/1806 (10.7%) of sextant cores (p = 0.72). The diagnosis of prostatic intraepithelial neoplasia (PIN) was suggested in 52/1133 (4.6%) of targeted cores and 63/1806 (3.5%) of sextant cores (p = 0.70). The diagnosis of atypical small acinar proliferation (ASAP) was suggested in 31/1133 (2.7%) of targeted cores and 47/1806 (2.6%) of sextant cores (p = 0.79). In contrast to the significantly increased detection of prostate cancer with targeted biopsy cores (OR = 2.0, p < 0.001), there was no statistically significant increase in the detection of prostatitis, PIN or ASAP with targeted biopsy.

Interobserver agreement for the independent assessments of ultrasound findings are reported in table 5. The value of kappa (κ) ranges from 0 (no agreement) to 1 (perfect agreement). κ was tabulated separately for the first blinded reader (experienced urologist without prior experience using contrast agents) and the second blinded reader (junior urologist with several months of training in contrast-enhanced imaging). The kappa values in table 5 demonstrate better interobserver agreement with the second blinded reader, with the best agreement using enhanced Doppler imaging (κ = 0.54-0.55). On re-review of discrepant cases some of the differences between readers were related to subjective differences in evaluation of the level of enhancement. Another important source of interobserver discrepancy was disagreement as to location of enhancing areas within the prostate. When reviewing the study on videotape, the blinded reviewer was less certain of the image location than the primary examining physician.

Discussion:

Persons with rising PSA are often subjected to multiple biopsy procedures. The positive biopsy rate on repeat sextant biopsy is approximately 19% after one initial negative biopsy, and drops to 8% after two negative biopsy procedures.[xxvii] The current study demonstrates that a targeted biopsy approach based upon contrast-enhanced sonography can improve the detection of prostate cancer relative to sextant biopsy (OR = 2.0, p < 0.001). Among patients with a previously negative biopsy, cancer was detected in 22/63 (35%: 95% CI: 23-48%) of subjects with one prior biopsy and in 14/71 (20%: 95% CI: 11-31%) of subjects with multiple prior biopsies. Thus, the positive biopsy yield with our contrast-enhanced targeted biopsy is superior to the positive biopsy yield reported in the literature for repeat sextant biopsy.

Studies of microvessel density within the prostate demonstrate a clear association of increased microvessel density with the presence of cancer,[xxviii] with metastases,[xxix] with the stage of disease[xxx,xxxi,xxxii] and disease-specific survival.[xxxiii,xxxiv] Quantitative assessment of microvascular density may actually provide important data to guide therapeutic decisions.[xxxv] However, the microvessels which proliferate in prostate cancer (10-30 microns) are below the resolution of conventional transrectal ultrasound. Microbubble ultrasound contrast agents represent one approach to visualize these microvessels. Recently developed ultrasound contrast agents have intravascular residence times of several minutes, pass through the pulmonary circulation, and may be used for parenchymal organ enhancement.[xxxvi,xxxvii] Recent clinical trials have demonstrated improved detection of prostate cancer with targeted biopsy based upon microbubble contrast agents.[xxxviii,xxxix,xl]

In order to enhance neovessels, contrast agents must pass into the microvascular circulation. Conventional gray scale and Doppler imaging destroy most contrast microbubbles before they reach the microvasculature. IHI provides an interscan period during which contrast material may traverse further into the capillary bed without being destroyed.[xx,xxi,xxii] When compared to continuous harmonic imaging, IHI provides a qualitatively different enhancement pattern based upon penetration of contrast agent into smaller vessels, with improved contrast enhancement of prostate cancer. Our clustered ROC analysis confirm a statistically significant advantage to IHI over baseline imaging for the identification of prostate cancer, but little advantage for IHI over continuous harmonic imaging (table 4).

Although clustered ROC analysis demonstrates a statistically significant improvement in the discrimination of prostate cancer with contrast-enhanced imaging, the areas under the ROC curve ($A_z = 0.60$-0.65) are only mildly superior to random chance ($A_z = 0.50$). The relatively low ROC A_z values for ultrasound detection of prostate cancer are not surprising in light of the poor interobserver agreement demonstrated in table 5. Improved kappa values for the second blinded reader ($\kappa = 0.35$-0.55) demonstrate the importance of training for interpretation of contrast-enhanced imaging.

Why is it so difficult to define prostate cancer with contrast enhanced imaging ? In contrast to the solitary, well defined spherical tumors present in many solid organs, prostate cancer is multifocal in 85% of cases, and the individual sites of tumor are often oblong and irregular in shape.[xli] Prostate cancer often grows along the capsule of the

prostate.[xlii] Furthermore, the normal radial vascular pattern of the prostate is often distorted by the presence of benign prostatic hyperplasia. For these reasons, the hypervascularity associated with prostate cancer may not present as a round mass, and may be difficult to differentiate from normal capsular vascularity.

If the discriminatory ability of contrast-enhanced ultrasound for prostate cancer is poor (ROC A_z < 0.65), why do targeted biopsy specimens double the positive yield for detection of cancer ? As demonstrated in figures 1 & 2, the higher positive biopsy yield of the targeted cores in this study confirms that areas of increased enhancement are more likely to contain a malignancy. On the other hand, the relatively low ROC areas (table 4) suggest that contrast enhanced imaging cannot adequately discriminate benign from malignant areas. The explanation is related to the many false positive sites of enhancement that reduce the specificity of contrast enhanced imaging.[xliii] In order to improve the discrimination of malignant from benign tissue, future efforts must concentrate on eliminating these areas of false positive enhancement.

Although targeted biopsy detected 11% (11/104) of cancers not found by the sextant approach, targeted biopsy failed to detect 20% (21/104) of cancers. Among 21 subjects whose cancer was not detected by targeted biopsy, 17 subjects had tumor in the gland apex on sextant biopsy. Chi-square analysis for trend demonstrates a significant decrease in cancer detection by targeted cores toward the apex of the prostate (p=0.006). In order to maximize cancer detection and minimize the number of biopsy cores, we suggest that a contrast-enhanced targeted biopsy strategy combined with additional "systematic" cores at the apex of the prostate.

Study Limitations:

ROC analysis was applied to evaluate our ability to discriminate benign from malignant prostatic tissue. The original plan was to use a consensus ultrasound rating of the examining physician and the blinded reader for this ROC analysis. However, during consensus readings it was often difficult to precisely demonstrate the spatial correspondence between the diagnostic portion of the study and the biopsy sites. It became obvious that the original readings of the primary examining physician at the time of the prostate biopsy procedure corresponded more closely to the selected biopsy sites. Based upon these considerations, we used the ultrasound interpretation provided by the examining physician for ROC analysis of contrast-enhanced ultrasound in the detection of prostate cancer.

The targeting of sextant cores to the most enhancing site within each sextant implies that the sextant cores in this study are not equivalent to a standard systematic sextant. A true comparison of targeted biopsy to the systematic sextant would require two independent examining physicians, one to perform the targeted biopsy and a second to perform the systematic sextant biopsy. The targeted sextant methodology selected for the current study would tend to increase the overlap between targeted and sextant biopsy cores, and should bias the comparison of targeted versus sextant biopsy toward a null result. Thus, our result for the comparison of targeted and sextant biopsy (OR = 2, $p < 0.001$) almost certainly underestimates the significant advantage of the targeted approach over a systematic sextant biopsy approach.

In actuality, the most vascular area within each sextant was almost always along the lateral margin of the prostate. Thus, the targeted sextant specimens were generally distributed in a similar pattern as the laterally directed sextant specimens. Nonetheless, given the potential sources of bias described above, the true difference in biopsy yield between the targeted approach and a systematic sextant approach is likely to be even larger than the difference reported in this study.

Why did we use a targeted sextant approach rather than a standard systematic sextant biopsy ? This study was designed to evaluate both the detection of prostate cancer and the discrimination of benign from malignant prostate tissue with contrast enhanced imaging. In order to evaluate the utility of contrast enhanced imaging, it was critical that the biopsy specimens should be taken from the sites of maximum enhancement. However, ROC analysis based upon targeted cores obtained at sites of increased enhancement would be biased by increased cancer detection secondary to multiple biopsy cores at enhancing sites. In order to avoid this potential bias, a targeted sextant approach was chosen with a single biopsy core corresponding to a single observer rating in exactly six locations in every prostate.

Pathology evaluation in this study is limited by the lack of correlation to whole mount prostatectomy specimens. Additional sites of malignancy within the prostate may not be detected by needle biopsy. It is likely that the diagnosis of cancer is missed in a minority of patients by needle biopsy.[xliv,xlv,xlvi] Nonetheless, since the primary goal of the study was to evaluate the detection of prostate cancer, we chose to correlate imaging

findings with the needle biopsy cores that are used for cancer detection. Furthermore, among the 301 subjects enrolled in this study, cancer was detected in 104. Since approximately 30% of patients diagnosed with cancer at our institution are treated with radical prostatectomy, the total number of patients with whole mount correlation would be approximately 31. Whole mount correlation for this subselected population would not provide information about cancer detection in the remaining 90% of our study patients.

We did not use transrectal injection of lidocaine in this study because of a fear that such an injection might alter the distribution of contrast enhancement in the prostate. Subsequent preliminary investigations in our department suggest that periprostatic injection of lidocaine does not visibly alter Doppler detection of blood flow around the prostate. However, based upon the current study we cannot comment on the use of transrectal anesthetic in patients who are evaluated with contrast-enhanced sonography.

Conclusion:

Intravenous infusion of a microbubble contrast agent provides sonographically visible enhancement of prostatic parenchyma, and can be used to target a biopsy procedure into areas of increased vascular flow. Among subjects with cancer, targeted cores were twice as likely to return a positive biopsy as compared to sextant cores (OR = 2.0, $p < 0.001$). With respect to the characterization of tissue as benign versus malignant, a statistically significant benefit was found for all methods of post-contrast IHI over baseline gray scale and Doppler imaging ($p < 0.05$). However, there was minimal advantage to IHI beyond that provided by continuous harmonic or Doppler imaging.

Targeted biopsy of the prostate based upon contrast-enhanced imaging does identify cancers that are not detected by conventional sextant biopsy. In order to maximize cancer detection with a minimum number of biopsy cores we recommend a contrast-enhanced targeted biopsy strategy with additional systematic cores distributed to the apex of the prostate.

Figure Captions:

Figure 1. 75 year old male with Gleason 8 cancer in the left mid gland.

(A) Baseline color Doppler image demonstrates a hypoechoic mass in the left mid-gland.

(B) Post contrast CHI demonstrate enhancement of the cancer (arrow).

(C) Post contrast IHI with 1.0second interscan delay demonstrates larger blush of tumor enhancement (arrow)

(D) Post contrast color Doppler demonstrates enhancement of tumor.

(E) Post contrast power Doppler demonstrates enhancement of tumor.

Figure 2. 78 year old male with Gleason 8 cancer in the left mid gland.

(A) Baseline color Doppler image demonstrates a small focal calcification in the left mid gland, but not other evidence of mass or cancer.

(B) Post contrast CHI demonstrates enhancement of the cancer (arrow)

(C) Post contrast IHI with 2.0second interscan delay demonstrates larger blush of subtle tumor enhancement (arrow)

(D) Post contrast color Doppler demonstrates enhancement of tumor.

(E) Post contrast power Doppler demonstrates enhancement of tumor.

Table 1. Postive biopsy rate in study patients as a function of number of previous biopsy procedures.

# previous biopsy procedures	# subjects	# cancers	% positive biopsy procedures
0	167	68	41%
1	63	22	35%
2	42	8	19%
3	16	3	19%
≥4	13	3	23%

Table 2. Cancers detected by sextant and targeted biopsy approaches

	Targeted +	Targeted -
Sextant +	72	21
Sextant -	11	197

Table 3. Gleason score distribution as a function of biopsy approach

	Gleason 5	Gleason 6	Gleason 7	Gleason 8	Gleason 9	Gleason 10
Sextant alone	1	15	3	1	1	0
Targeted alone		10	1			
Total	2	57	26	14	3	2

Table 4. Areas under the curve (A_z)for clustered ROC analysis using different ultrasound imaging techniques.

Ultrasound Imaging Technique	Area under the ROC curve (Az)
Pre-contrast gray scale (baseline)	0.58
Pre-contrast color Doppler (baseline)	0.53
Pre-contrast power Doppler (baseline)	0.58
Post-contrast gray scale harmonic imaging	0.62
Intermittent gray scale harmonc imaging (0.2s)	0.64
Intermittent gray scale harmonc imaging (0.5s)	0.63
Intermittent gray scale harmonc imaging (1.0s)	0.65
Intermittent gray scale harmonc imaging (2.0s)	0.61
Contrast-enhanced color Doppler	0.60
Contrast enhanced power Doppler	0.62

Table 5. Interobserver agreement between the examining physician and a blinded reader as expressed by a quadratic kappa.

Ultrasound Imaging Technique	Kappa: examining physician vs. reader #1	Kappa: examining physician vs. reader #2
Pre-contrast gray scale (baseline)	0.19	0.34
Pre-contrast color Doppler (baseline)	0.28	0.46
Pre-contrast power Doppler (baseline)	0.28	0.50
Post-contrast gray scale harmonic imaging	0.17	0.53
Intermittent gray scale harmonc imaging (0.2s)	0.18	0.47
Intermittent gray scale harmonc imaging (0.5s)	0.19	0.49
Intermittent gray scale harmonc imaging (1.0s)	0.16	0.46
Intermittent gray scale harmonc imaging (2.0s)	0.07	0.35
Contrast-enhanced color Doppler	0.12	0.54
Contrast enhanced power Doppler	0.09	0.55

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